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NOTES ON THE GROWTH AND ULTRASTRUCTURE OF *BIDDULPHIA LAEVIS* EHR. (BACILLARIOPHYCEAE) IN THE MAUMEE RIVER, OHIO[1]

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ABSTRACT. Masses of the chain-forming diatom *Biddulphia laevis* Ehr. were observed in the Maumee River in the summer of 1981. Standing crop of this diatom was determined at different thalli portions of its green algal substrate, *Cladophora glomerata* (L.) Kutz. Ultrastructural observations of valve morphology with SEM indicate this species is typical of other biddulphioid diatoms, except in the structure of the ocellus-like process, which appears to be intermediate between a typical rimless pseudocellus and the thickened rim of an ocellus. Attachment of *B. laevis* to *Cladophora* and the zigzag filamentous nature of the chains was observed with SEM and noted to be similar to that of marine centric diatoms of the same type.

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INTRODUCTION

Biddulphia laevis Ehr., a relatively large centric diatom, is most commonly associated with marine or brackish water. Cholnoky (1968) characterizes this taxon to be oligo-to-mesohalobous while Simonsen (1962) considers it to be mesohalobous.

This diatom has been reported from a number of rivers in the United States. In most instances, these occurrences have been from arid regions in rivers with relatively high total dissolved solids. Czarnecki and Blinn (1978) reported *B. laevis* in Diamond Creek and Elves Chasm, 2 tributaries of the Colorado River. They observed it is primarily epilithic and seems to prefer current. Crayton and Sommerfield (1979) encountered this diatom as a dominant member of the phytoplankton in the same 2 streams. In Elves Chasm they observed 6,500 cells/liter. They correlated distribution of this diatom with high nitrate levels in the water. Reinke (1979) reported *B. laevis* from 2 small creeks in Kansas but did not comment on abundance or environmental conditions. The first report of this diatom from the Laurentian Great Lakes came in 1981 from Wujek and Welling who collected *B. laevis* from Lake Michigan. They refer to it as a halophil and comment that it probably is indicative of increasing near shore chloride levels.

The only reports of *B. laevis* in Ohio have been by Hirsch and Palmer (1958) in the Scioto River and by Collins and Kalinsky (1977) in Big Darby Creek. Neither report offers details of the growth or microhabitat of this diatom. When con-

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spicuous growth of *B. laevis* began appearing in the Maumee River (Wood County) in the fall of 1981, it provided an opportunity to learn more about the microhabitat, attachment and growth of this species.

METHODS AND MATERIALS

Large brown masses of the diatom *Biddulphia laevis* were observed in late August 1981 in the Maumee River, approximately 1.5 km upstream from Waterville, Ohio (fig. 1). Collections were taken from portions of older and younger *Cladophora glomerata* thalli in riffle areas of the river where masses of the diatom were encountered. A bar-clamp sampler (Gale 1975) was employed to sample specific substrate surface area. Three replicate counts were made on each sample by examining one-half of a standard Palmer counting cell.

Material was collected for ultrastructural observations with light and scanning electron microscopy. Material to be viewed with the light microscope was "cleaned" by boiling in nitric acid for 2 hours, rinsed several times in distilled water and air-dried onto coverslips. The dried coverslips were permanently mounted onto standard microscope slides with Hyrax. Slides were viewed with a Leitz Dialux-20 light microscope. Collections taken for scanning electron microscopy were field-fixed with 2.5% phosphate-buffered glutaraldehyde for 2 hours, then osmicated with 2.0% osmium tetroxide for 2 hours. Following fixation, material was washed in phosphate buffer (pH 7.2), dehydrated through an ethanol series, critical-point dried with CO_2, then sputter-coated with approximately 20 nm of a gold-palladium source. Material cleaned for light microscopy was also prepared for SEM. Material was air-dried on a coverslip which was attached to an aluminum stub with tube coat then coated with approximately 20 nm of a gold-palladium source. Material was viewed on a Hitachi HHR-2S scanning electron microscope at an operating voltage of 20 KV and at a tilt angle of approximately 20°.

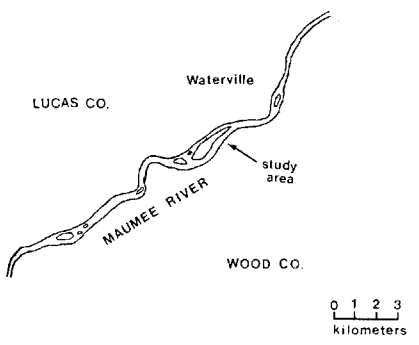

FIGURE 1. Map of Maumee River in the vicinity of Waterville, Ohio.

Water samples were taken from the study site on 21 August 1981 for estimation of total dissolved solids. Two water samples of 100 ml were filtered through Millipore filters (pore size 0.45 μm) and were processed according to standard methods for determination of total dissolved solids as filtrable residue (American Public Health Association 1971).

RESULTS

Measurement of total dissolved solids from the Maumee River, 21 August 1981 yielded a value of 600 ppm. This figure appears to be similar to reports of other water systems in the area; for example values of 532 ppm for Maumee Bay (Fraleigh et al. 1979) and 964 ppm for the Sandusky River (Martin 1976) have been reported.

Colonies of *Biddulphia laevis* were observed throughout the study area, however best development occurred on older filaments of *Cladophora glomerata*. Densities of *B. laevis* ranged from 9 to 3,924 cells/mm^2, with younger thalli averaging 13 cells/mm^2 and older thalli averaging 2,816 cells/mm^2.

Ultrastructural observations made with light and scanning electron microscopy support previously documented observations of *Biddulphia laevis* made with the light microscope. Terminology of ultrastructural components follows that of Anonymous (1975) and Ross et al. (1979). The population of *B. laevis* observed in the Maumee River ranged in valve diameter from 40 to 60 μm, and possessed 14–16 areolae in 10 μm. These observations fall within the ranges noted by Hustedt (1930) and Cleve-Euler (1951). Two ocelli, 2–4 central labiate processes and short peripheral spines were evident in the light microscope (fig. 2A and B). Observation of the external valve face with SEM shows it supporting many small granules and spines in both cleaned and uncleaned material (fig. 2C and D); however, internally the openings of the areolae are much clearer (fig. 2E). Labiate processes, peripheral spines and the ocelli-like processes bordered by a thin distal rim and comprised of many porelli are evident on the external valve face (fig. 2F). Examination of the in-

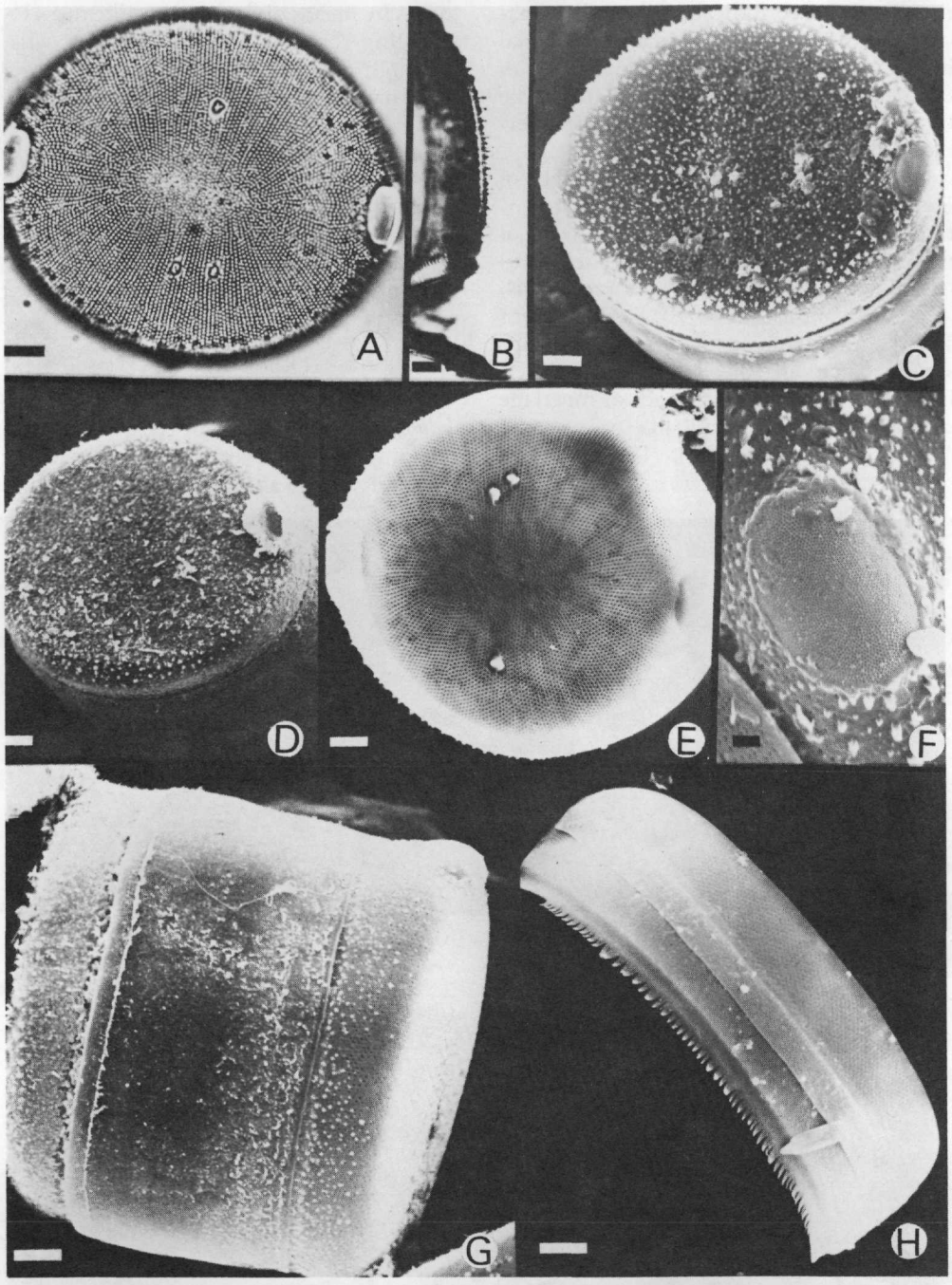

FIGURE 2. A. Light micrograph of valve face. B. Light micrograph of girdle view showing peripheral spines. C. Scanning electron micrograph of cleaned external valve face. D. SEM of uncleaned valve face. E. SEM of internal valve face. F. SEM close-up of ocellus, showing peripheral rim and porelli. G. SEM of girdle view. H. SEM of girdle band. (Bar in A–E, G, H = 5 μm. In F = 1 μm.)

ternal valve face indicates the stalked nature of the labiate processes and the radial nature of the areolae. A girdle view of *B. laevis* reveals a wide, punctate girdle band (fig. 2G) which has peripheral finger-like projections (fig. 2H).

Like other species of *Biddulphia*, cells of *B. laevis* were arranged in zigzag filamentous colonies (fig. 3A). Previous reports of the colonies of this diatom have described filaments of relatively short length (Czarnecki and Blinn 1978). In the present study, some filaments of *B. laevis* were observed to contain 50 cells. The cells of filaments were connected by pads of mucilage associated with the ocelli-like processes of adjoining cells (fig. 3B). The filaments were attached to *Cladophora glomerata* filaments by means of a mucilage-like mass resembling the pads located between cells, although the anchoring mass was larger and more complex in its appearance (fig. 3C). Cells of *B. laevis* dislodged from the attachment areas revealed the masses to consist of a depressed ovoid center and an elevated circular area which surrounds the center (fig. 3D). It has not been determined if the entire structure assists in anchoring *B. laevis* to the *Cladophora* substrate.

DISCUSSION

Ultrastructural observations of *Biddulphia laevis* by light and scanning electron microscopy reveal this diatom to be morphologically similar to other members

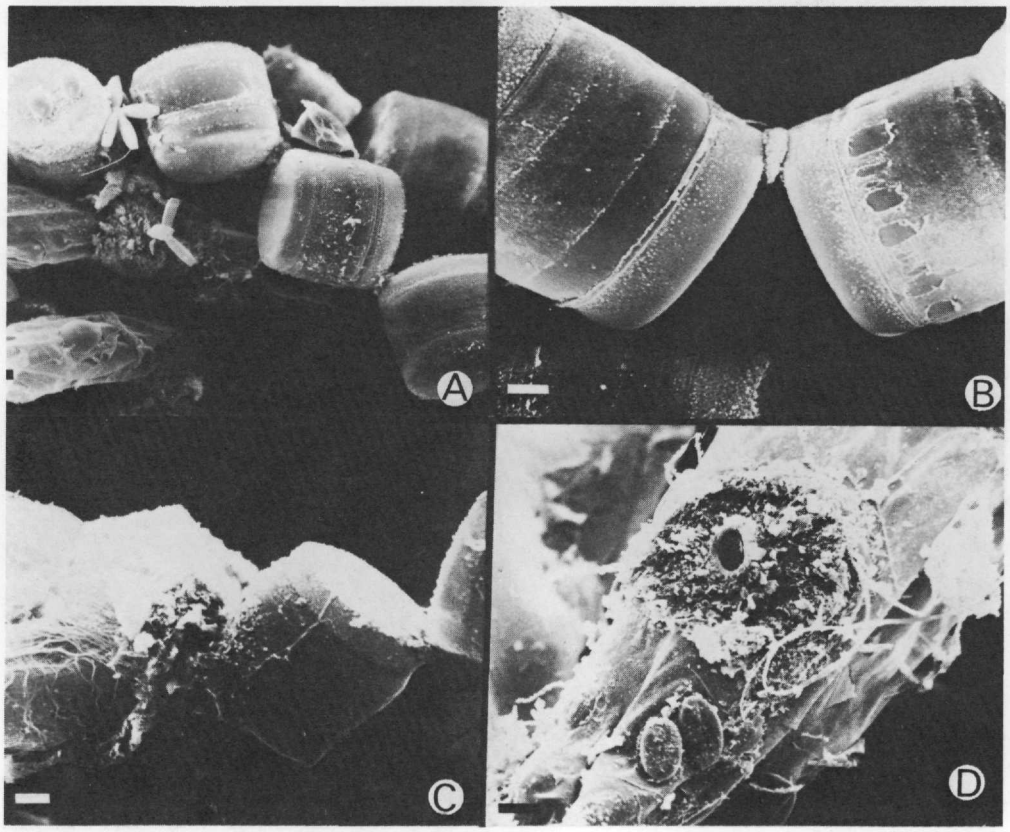

FIGURE 3. A. SEM of *Biddulphia* filament around *Cladophora glomerata*. B. SEM of mucilage pad between two connected cells. C. SEM of attached *Biddulphia* filament on *Cladophora*. D. SEM of attachment site. (Bar in A–D = 10 μm.)

of the genus *Biddulphia* (Helmcke and Krieger 1963, Helmcke et al. 1974). However, the ocellus-like process, with its distinct rim, appears to be a true ocellus rather than pseudocellus which is characteristic of the subfamily Biddulphinaea of which the genus *Biddulphia* is a member (Simonsen 1979). The rim of the ocellus in *B. laevis* is quite reduced compared to those observed in other taxa (Ross and Sims 1971, 1972) and may represent a structure intermediate between an ocellus and a pseudocellus, as those terms are currently applied (Ross et al. 1979). Although Simonsen (1979) has based the distinction between 2 subfamilies of the family Coscinodiscaceae on the presence of either an ocellus or a pseudocellus, he indicates a relationship between the 2, with the pseudocellus being the progenitor to the ocellus. The presence of an ocellus in *B. laevis* may suggest the 2 structures now used to separate taxa may be of a single type which grades from a pseudocellus to an ocellus. Functionally the 2 structures are similar (Simonsen 1979).

Previous reports of large populations of *B. laevis* from inland rivers have been qualitative (Hirsch and Palmer 1958) or based on planktonic sampling methods (Crayton and Sommerfield 1979). The large numbers of *B. laevis* observed epiphytic on *Cladophora glomerata* in the present study, and previous reports of its epilithic habit (Czarnecki and Blinn 1978) suggest this diatom is primarily an attached form which may become dislodged into the plankton. The large numbers of *B. laevis* observed in riffle areas of the Maumee River suggest this diatom has a preference for faster flowing water. Czarnecki and Blinn (1978) have characterized this diatom as a rheophil. The microhabitat of *B. laevis* in the Maumee River appears to consist of *Cladophora glomerata* filaments, with best development occurring on older thalli in riffle areas during late summer when total dissolved solids may be high. The larger standing crop of *B. laevis* observed on older *Cladophora* thalli probably was due to a longer growing time, allowing the diatom filaments to increase in length.

The method of attachment to a substrate via a mucilage-like mass is similar to that of the marine diatoms *Isthmia nervosa, I. enervis, Melosira borreii* (Smith 1853) and other *Biddulphia* species (Hendey 1964). The structure of the attaching mass is notable. Observations of the pad suggest *B. laevis* may secrete an abundance of a mucilage-like substance for attachment which may eventually cover the ocellus of the cell, producing a characteristic central ovoid depression. The mass of material also may be added to by debris which may collect on the mucilage. However, this material has not been observed on mucilage pads situated between cells.

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